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Prelabeling of Chimeric Monoclonal Antibody L6 with ^{90}Yttrium- and ^{111}Indium-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA) Chelates for Radioimmunodiagnosis and Therapy[1]

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Abstract

^{90}Y and ^{111}In have been attached to chimeric monoclonal antibody L6 with a bifunctional chelating agent (DOTA-peptide isothiocyanate). The bifunctional chelating agent was prelabeled with either radiometal and then conjugated to the antibody. Studies in human patients showed excellent ^{111}In single-photon emission computed tomography images of breast cancer lesions 24 h after injection.

Introduction

The human/mouse chimeric antibody L6 recognizes a cell surface antigen highly expressed on lung, breast, colon, and ovarian carcinomas (1). Radioiodinated L6 is being used in a Phase I clinical trial for therapy of metastatic breast cancer (2). We are working to extend the use of this antibody to include other radionuclides for imaging and therapy.

Metallic radioisotopes such as ^{111}In, ^{90}Y, ^{67}Cu, $^{99\,m}$Tc, and ^{68}Ga are primary candidates for labeling mAbs due to their favorable physical properties (3, 4). Macrocyclic bifunctional chelating agents have been developed to tag mAbs with radiometals for *in vivo* diagnosis and therapy (5–11); in particular, antibodies labeled with DOTA[3] derivatives incorporating ^{90}Y and ^{111}In have shown excellent kinetic stability under physiological conditions (6, 9, 11, 12). However, the slow formation of yttrium-DOTA complexes presents a technical problem that can lead to low radiolabeling yields unless conditions are carefully controlled. Prelabeling, in which the bifunctional chelating agent is first radiolabeled and then conjugated to the mAb, provides an alternative (13). The prelabeling approach permits use of a large excess of bifunctional chelating agent to achieve a high chelation yield quickly and uses a rapid purification method to remove unlabeled reagent before conjugation to the antibody. Here we report the first human studies using chimeric antibody L6 prelabeled with a peptide-linked DOTA derivative containing ^{111}In (or ^{90}Y).

Materials and Methods

The labeling procedure is outlined in Fig. 1. The bifunctional chelating agent DOTA-peptide isothiocyanate (Fig. 1) was prepared by the method described recently (11). Carrier-free $^{90}YCl_3$ (Westinghouse; 75–250 mCi) or $^{111}InCl_3$ (Amersham Medi-Physics; 50–70 mCi) in 0.05 M HCl was dried on a hot plate at 60–80°C under a gentle stream of filtered nitrogen gas (Millipore) and 100 μl of 20 mM DOTA-peptide isothiocyanate in 0.2 M tetramethylammonium acetate (pH 5.0) was added. The mixture was incubated at 37°C for 30 min, followed by addition of 25 μl of 50 mM DTPA in 0.1 M tetramethylammonium acetate (pH 6.0) for 15 min at room temperature to complex any remaining free yttrium (or indium).

An anion-exchange column had been prepared by filling a 1-ml disposable tuberculin syringe with 500 μl of DEAE cellulose resin (1 meq/dry g; Sigma Chemical Co.) in the acetate form. The reaction solution was loaded onto the column, and the desired product eluted with H_2O. Most of the radioactive ^{111}In- or ^{90}Y-DOTA-peptide isothiocyanate was recovered in the first four 100-μl fractions.

The eluted ^{111}In- or ^{90}Y-DOTA-peptide isothiocyanate solution was mixed with approximately 20 mg of chimeric mAb L6 (ChL6; 340 μl, 59 mg/ml; Oncogen/Bristol-Myers; Ref. 14) in 0.1 M tetramethylammonium phosphate (pH 9.0). The pH was adjusted to 9.5 using aqueous 2.0 M triethylamine. The mixture was incubated at 37°C for 1 h, and the radioimmunoconjugate ^{111}In- or ^{90}Y-DOTA-peptide-thiourea-ChL6 was isolated using a Sephadex G-25 gel-filtration column.

The radiochemical purity of both ^{90}Y- and ^{111}In-labeled immunoconjugates was determined to be >95% by gel filtration HPLC, cellulose acetate electrophoresis, and silica gel TLC (15). A solid-phase RIA (16) was performed using ^{125}I-labeled ChL6 as a standard. The immunoreactivity of ^{111}In- or ^{90}Y-DOTA-peptide-thiourea-ChL6 was indistinguishable from ^{125}I-labeled antibody.

Results and Discussion

DOTA-peptide isothiocyanate forms electrically neutral complexes with trivalent metals such as indium or yttrium. All the other important species in the chelation reaction mixture, such as excess chelating agents, complexes containing *divalent* metals, and DTPA complexes, are negatively charged. Thus, the DOTA-peptide isothiocyanate complexes with trivalent metals can be filtered quickly through an appropriately designed anion-exchange column in H_2O to separate them from anionic species.

In the chelation step, the yield after anion-exchange purification was 43–61% of the starting radioactivity when the radiometal was ^{111}In but was lower and more variable (9–41%) when the radiometal was ^{90}Y. Particularly for ^{90}Y solutions, the levels of impurities appear to vary with each batch of carrier-free radiometal and include both other metals and species that react with the isothiocyanate group. The identity of these impurities is difficult to determine, but most common metal contaminants are divalent ions. Prelabeling deals with the impurity problem by using a large excess of chelating agent and then removing the excess. This is preferable to using a large excess of chelate-tagged mAb conjugate, which cannot be fractionated later to remove unwanted contaminants. Prelabeling does not eliminate trivalent metal complexes from the product, and conjugation yields are decreased by impurities in the radionuclide solution that react with the isothiocyanate group (*e.g.*, amines).

In the conjugation step, a high concentration of mAb is desired, so that each chelate isothiocyanate will frequently encounter amino groups with which to react. At the chosen conjugation conditions (1 h incubation at 37°C, pH 9.5; mAb ≥ 50 mg/ml), the conjugation yield is almost quantitative when based on the actual amount of intact DOTA-peptide isothiocyanate added, as determined by HPLC. The final yields and specific activities of the isolated radioimmunoconjugates used in these experiments were 10–45% and 0.7–2.3 mCi/mg for ^{111}In-DOTA-peptide-thiourea-ChL6 and 2–34% and 0.2–2.4 mCi/mg for ^{90}Y-DOTA-peptide-thiourea-ChL6.

In the conventional labeling method, the ratio of attached chelates to mAb is usually >1 in order to provide enough chelating groups for

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[3] The abbreviations used are: DOTA, 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid.

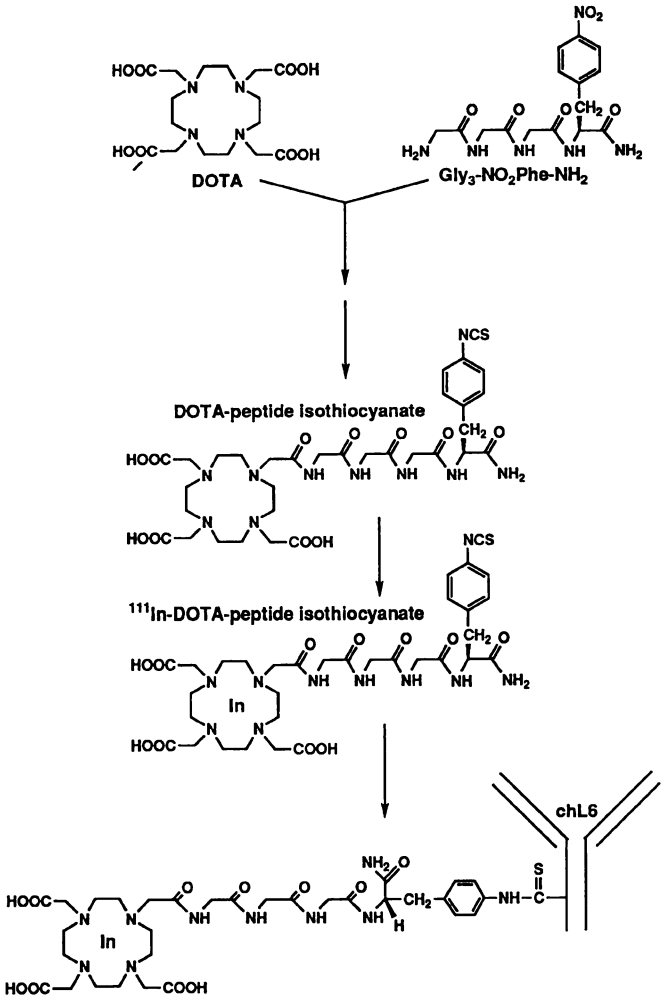

Fig. 1. Outline of the chemistry involved in conjugating antibody ChL6 with ^{111}In- or ^{90}Y-DOTA-peptide isothiocyanate.

a good radiolabeling yield. However, the chelates that are actually used for labeling comprise less than 5% of the total attached chelates on the mAb. The excess chelating groups may affect the biological properties of mAbs, *e.g.,* by inducing an immune response (17), and impure metal solutions may require large amounts of immunoconjugate. With prelabeling, a far smaller number of chelates becomes attached to the mAb, but practically all are radiolabeled; thus, the number of modified mAbs is significantly reduced, and the number of multiply-modified mAbs is essentially zero. The radiolabeled antibodies are fully immunoreactive and are expected to have more favorable biological properties, including less immunogenicity. Another reason for using DOTA-peptide is to reduce the accumulation of radioactivity in the liver by introducing a cleavable linker between the chelate and the mAb (11).

To examine the properties of the conjugate *in vivo*, ^{111}In- or ^{90}Y-DOTA-peptide-thiourea-ChL6 was injected into HBT tumor-bearing nude mice (18) for organ distribution and tumor uptake studies, with favorable results (13).

Based on these data from nude mice, a human investigative new drug application was approved for pharmacokinetic and therapy studies. Quantitative imaging studies with pharmacokinetic data acquisition have been performed in three patients; images acquired between 1 and 5 days after the injection of 4 to 10 mCi of ^{111}In-DOTA-peptide-thiourea-ChL6 demonstrated excellent tumor targeting of the metastatic breast cancer (Fig. 2). This uptake correlated with the small

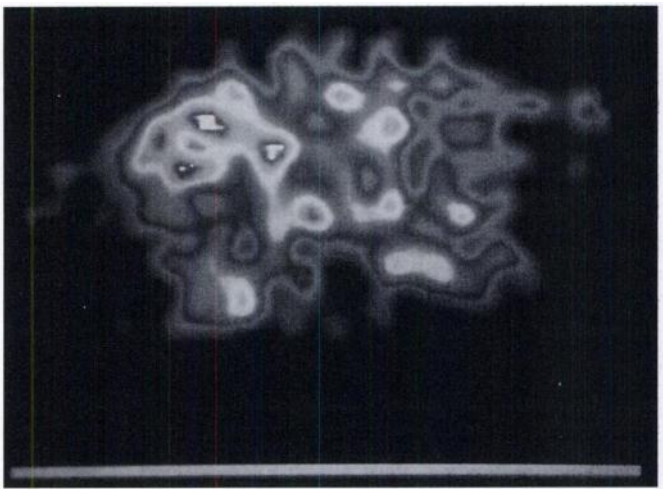

Fig. 2. Single-photon emission computed tomography image of the mid-chest area of a patient with metastatic breast cancer, 72 h after injection of 4 mCi of ^{111}In-DOTA-peptide-thiourea-ChL6. Uptake in metastatic lesions are seen in the anterior and posterior mediastinal lymph nodes, right anterior and bilateral posterior ribs, and a large right anterior tumor mass in lung, which has a central area void of uptake.

deposits of disease found on CT imaging in these regions. Blood clearances of the ^{111}In- and ^{90}Y-DOTA-peptide-thiourea-ChL6 were comparable and within the range of ^{131}I blood clearances demonstrated previously in patients (2, 19). Tumor dosimetry for ^{90}Y as DOTA-peptide-thiourea-ChL6 was extrapolated from the uptake of ^{111}In. Radiation doses calculated for ^{90}Y to the metastatic tumors were 4 to 8 times those calculated from ^{131}I ChL6 (rads/mCi).

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References

1. Hellström, I., Horn, D., Linsley, P. S., Brown, J. P., Brankovan, V., and Hellström, K. E. Monoclonal mouse antibodies raised against human lung carcinoma. Cancer Res., *46:* 3917–3923, 1986.
2. DeNardo, S. J., Mirick, G. R., Kroger, L. A., O'Grady, L. F., Erickson, K. L., Yuan, A., Lamborn, K. R., Hellström, I., Hellström, K. E., and DeNardo, G. L. The Biologic Window For Chimeric L6 Radioimmunotherapy. Cancer (Phila.), *73*(Suppl.)*:* 1023–1032, 1994.
3. Liu, Y., and Wu, C. Radiolabeling of monoclonal antibodies with metal chelates. Pure & Appl. Chem., *63:* 427–463, 1991.
4. Wessels, B. W., and Rogus, R. D. Radionuclide selection and model absorbed dose calculations for radiolabeled tumor associated antibodies. Med. Phys., *11:* 638–645, 1984.
5. Moi, M. K., Meares, C. F., McCall M. J., Cole, W. C., and DeNardo, S. J. Copper chelates as probes of biological systems: stable copper complexes with a macrocyclic bifunctional chelating agent. Anal. Biochem., *148:* 249–253, 1985.
6. Moi, M. K., Meares, C. F., and DeNardo, S. J. The peptide way to macrocyclic bifunctional chelating agents: synthesis of 2-(*p*-nitrobenzyl)-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid and study of its Yttrium(III) complex. J. Am. Chem. Soc., *110:* 6266–6267, 1988.
7. Cox, J. P. L., Craig, Andrew, S., Helps, L. M., Jandowski, K. J., Parker, D., Eaton, M. A. W., Millican, A. T., Millar, K., Beeley, N. R. A., and Boyce, B. A. Synthesis of C- and N-functionalised derivatives of 1,4,7-triazacyclononane-1,4,7-triyltriacetic acid (NOTA), 1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetrayltetra-acetic acid (DOTA), and diethylenetriaminepenta-acetic acid (DTPA): bifunctional complexing agents for the derivatisation of antibodies. J. Chem. Soc. Perkin Trans., *1:* 2567–2576, 1990.
8. Parker, D. Tumor targeting with radiolabeled macrocycle-antibody conjugates. Chem. Soc. Rev., *19:* 271–291, 1990.
9. Meares, C. F., Moi. M. K., Diril, H. Kukis, D. L., McCall, M. J., Deshpande, S. V., DeNardo, S. J., Snook, D., and Epenetos, A. A. Macrocyclic chelates of radiometals for diagnosis and therapy. Br. J. Cancer Suppl., *10:* 21–26, 1990.
10. Gansow, O. A. Newer approaches to the radiolabeling of monoclonal antibodies by use of metal chelates. Nucl. Med. Biol., *18:* 369–381, 1991.
11. Li, M., and Meares, C. F. Synthesis, metal chelate stability studies, and enzyme digestion of a peptide-linked DOTA derivative and its corresponding radiolabeled immunoconjugates. Bioconjugate Chem., *4:* 275–283, 1993.
12. Deshpande, S. V., DeNardo, S. J., Kukis, D. L., Moi, M. K., McCall, M. J., DeNardo,

G. L., and Meares, C. F. Yttrium-90-labeled monoclonal antibody for therapy: labeling by a new macrocyclic bifunctional chelating agent. J. Nucl. Med., *31:* 473–479, 1990.

13. Li, M., Meares, C. F., Zhong, G. R., Miers, L., Xiong, C. Y., and DeNardo, S. J. Labeling monoclonal antibodies with ^{90}Yttrium- and ^{111}Indium-DOTA chelates: a simple and efficient method. Bioconjugate Chem., *5:* 101–104, 1994.
14. Fell, H. P., Gayle, M. A., Yelton, D., Lipsich, L., Schieven, G. L., Marken, J. S., Aruffo, A., Hellström, K. E., Hellström. I., and Bajorath, J. Chimeric L6 anti-tumor antibody: genomic construction, expression, and characterization of the antigen binding site. J. Biol. Chem., *267:* 15552–15558, 1992.
15. Meares, C. F., McCall, M. J., Reardan, D. T., Goodwin, D. A., Diamanti, C. I., and McTigue, M. Conjugation of antibodies with bifunctional chelating agents: isothiocyanate and bromoacetamide reagents, methods of analysis, and subsequent addition of metal ions. Anal. Biochem., *142:* 68–78, 1984.
16. DeNardo, S. J., Peng, J-S., DeNardo, G. L., Mills, S. L., and Epstein, A. L. Immunochemical aspects of monoclonal antibodies important for radiopharmaceutical development. Nucl. Med. Biol., *13:* 303–310, 1986.
17. Kosmas, C., Snook, D., Gooden, C. S., Courtenay-Luck, N. S., McCall, M. J., Meares, C. F., and Epenetos, A. A. Development of humoral immune responses against a macrocyclic chelating agent (DOTA) in cancer patients receiving radioimmunoconjugates for imaging and therapy. Cancer Res., *52:* 904–911, 1992.
18. Hellström, I., Horn, D., Linsley, P., Brown, J. P., Brankovan, V., and Hellström, K. E. Monoclonal mouse antibodies raised against human lung carcinoma. Cancer Res., *46:* 3917–3923, 1986.
19. DeNardo, S. J., O'Grady, L. F., Macey, D. J., Kroger, L. A., DeNardo, G. L., Lamborn, K. R., Levy, N. B., Mills, S. L., Hellström, I., and Hellström, K. E. Quantitative imaging of mouse L-6 monoclonal antibody in breast cancer patients to develop a therapeutic strategy. Nucl. Med. Biol., *18:* 621–631, 1991.

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